

in the NH₂- and -COOH terminal regions. These changes lead to frame shifts that are not compatible with a conserved ORF, therefore an assumption is that the sequence obtained from the 2.7 kb fragment represents a pseudogene (*FUT2P*). After subcloning of ETHs3 BamHI digests, the hybridizing sequences contained in the 8.2 kb EcoRI fragment were 5 identified. The sequence of the subclones obtained represents a 1023 bp ORF and is 85% identical at the nucleotide- and 83%- identical at the amino acid level to the human *FUT2* sequence. Many differences in the NH₂- and -COOH terminal regions were observed between the porcine *FUT2* sequence and the *FUT2P* sequence derived from the 2.7 kb fragment. The predicted amino acid sequence corresponds to the partially determined 10 amino acid sequence of the porcine *Secretor* enzyme (Thurin and Blaszczyk-Thurin, 1995). The porcine *FUT1*, *FUT2*, and *FUTP* sequences obtained were submitted to GenBank and have accession numbers U70883, U70881 and U70882, respectively. The *FUT1* and *FUT2* genes have highly homologous sequences. This has to be considered in, for example, primer development. Furthermore, *FUT1* and *FUT2* enzyme activity 15 need to be differentiated in further studies.

Example 6: Identification of M307 and M857 Mutations and Characterization of M307

DNA was isolated from porcine nucleated cells according to standard procedures. 20 Direct sequencing of porcine *FUT1* and *FUT2* sequences and their flanking regions in animals of different *ECF18R* genotypes (*Bb*, *bb*) resulted in the identification of two G-A transitions at positions 307 and 857 (termed *M307* and *M857*, respectively) of the *FUT1* ORF. The *M307* transition eliminates a restriction site for the enzyme CfoI. Amplification of DNA isolated from porcine nucleated cells was performed according 25 to standard procedures with primers P6 and P11 (3 min at 95°C, 30 cycles of 30 sec at 95°C, 30 sec at 56°C and 30 sec at 72°C, followed by a 7 min final extension at 72°C) followed by CfoI digestion and separation on a 3% agarose gel resulted in a restriction fragment length polymorphism (RFLP). Homozygous *M307^{AA}* animals showed 2 bands (93- and 328-bp fragments). Homozygous *M307^{GG}* animals showed 87-, 93-, an 241-bp 30 fragments. Heterozygous animals showed all four fragments.

Example 7: Characterization of Mutation M857

The M857 mutation is a transition that eliminates an AciI site. Primer PBEST was designed to mismatch two additional AciI sites at positions 866 and 872. PCR with - - primers P7 and PBEST (3 min at 95°C, 30 cycles of 30 sec at 95°C, 30 sec at 56°C and - -